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(71) Applicant *(for all designated States except US)*: **EFA SCIENCES LLC.** [US/US]; Suite 266, 1420 Providence Highway, Norwood, MA 02062 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **DAS, Undurti, Narasimha** [IN/US]; EFA Sciences LLC., Suite 266, 1420 Providence Highway, Norwood, MA 02062 (US).

(74) Agent: **NATH, Rama, B.**; 42 Sudbury Road, Ashland, MA 01721 (US).

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(54) Title: METHODS FOR TREATING CELL PROLIFERATIVE DISORDERS INCLUDING CANCER

(57) Abstract: A method of treating a cell proliferative disorder including types of cancer, causes inhibition of blood supply to the cancerous region, eg., a tumor, by administering an admixture of a predetermined quantity of a polyunsaturated fatty acid in the form of a solution of at least one polyunsaturated fatty acid together with optionally a predetermined anticancer drug, a cytokine and an oily lymphographic agent. In a preferred form, the admixture is injected intra-arterially through a catheter into an artery which provides the major blood supply to the tumor, the artery being proximate to the tumor. The solution of the polyunsaturated fatty acid may comprise an essential fatty acid(s) metabolite in salt form, eg., a lithium salt for non-glioma type of tumor, and a sodium/potassium salt for treating glioma. The polyunsaturated fatty acid content in the admixture may be in the range of 0.5 mg to 50 gm. The cytokine (lymphokine) may be tumor necrosis factor and/or interferon. The admixture may be administered orally or parenterally or by methods which are selective combinations, eg., intra-arterial, oral and parenteral. Examples given of cancers treated include hepatoma, giant cell tumor of the bone and glioma. The invention by cutting off blood supply to the tumor region causes massive necrosis of the cancerous cells, and prevents angiogenic activity in the tumor region.

METHODS FOR TREATING CELL PROLIFERATIVE DISORDERS INCLUDING CANCER

Field of Invention:

This invention relates to methods for treating cell proliferative disorders including types of cancer.

Background of the Invention:

Efforts have been made over the past several years in inventing methods of treatment of cell proliferation disorders, more specifically types of cancers including glioma.

Acronyms used in this invention:

TNF = Tumor necrosis factor.

IFN = Interferon.

EFAs = Essential fatty acids.

PUFAs = Polyunsaturated fatty acids.

LA = Linoleic acid.

GLA = Gamma-linolenic acid.

DGLA = Dihomo-GLA.

AA = Arachidonic acid.

ALA = Alpha-linolenic acid.

EPA = Eicosapentaenoic acid.

DHA = Docosahexaenoic acid.

CT = Computer tomography.

MRI = Magnetic resonance imaging

Designing methods to deliver drug(s) selectively to the target tissue is an established goal in the field of medicine. In general, it is always desirable to deliver a drug or a combination of drugs or derivatives of drugs or a group of drugs/compounds selectively to its target, so that dosage and, consequently, side effects can be reduced or prevented without compromising on the ultimate desired action on the target tissue or organ. This is particularly important in the case of anti-cancer drugs or agents because achieving therapeutic doses or effective doses for the treatment of cancer is often limited by the toxic side effects of the anti-cancer agent or drug on normal, healthy tissue/organ.

One method that can be adopted to overcome the toxic side-effects of anti-cancer drugs or agents is by the administration of compounds or agents that can prevent their side effects either along with it or in combination with it. This method of approach to treat cancer assumes added significance if the anti-cancer drugs or agents can be combined or conjugated to compounds which by themselves also have significant killing action on the cancer cells.

It is seen from the available literature that polyunsaturated fatty acids (PUFAs) have been known to have cytotoxic properties towards tumor

cells in vitro. However, the conditions existing during in vitro situations influencing the action of PUFAs alone or in conjunction with other additives, on tumor cells can not be replicated in living beings for various reasons.

Essential fatty acids (EFAs) are natural compounds that are present in human diet but can not synthesized by the body. Since they are not formed in the body but are necessary for normal health they are called as essential fatty acids. These EFAs belong to the class of fatty acids called as polyunsaturated fatty acids (PUFAs). PUFAs are classified in accordance with a short hand nomenclature which designates the number of carbon atoms present (chain length), the number of double bonds in the chain and the position of double bonds nearest to the terminal methyl group. The term n-x is used to describe the position of the double bond nearest to the methyl group. There are 4 independent families of PUFAs, depending on the parent fatty acid from which they are synthesized. They are:

a. n-6 series derived from linoleic acid (LA, 18:2)

b. n-3 series derived from alpha-linolenic acid (ALA, 18:3)

c. n-7 series derived from palmitoleic acid

d. n-9 series derived from oleic acid (OA, 18:1).

The parent fatty acids of the n-3 and n-6 series can not be synthesized by the mammals hence, they have to be obtained in the diet and for this reason are referred to as essential fatty acids. It should be emphasized that all EFAs are PUFAs but not all PUFAs are not EFAs. Of the 4 PUFA families, n-3 and n-6 are the most important since, they and their metabolic products have diverse biological actions.

It is believed that both LA and ALA are metabolised by the same set of enzymes (see Figure1). LA is converted to GLA by the action of the enzyme, delta-6-desaturase which in turn is elongated to form dihomo-GLA (DGLA), the precursor of 1 series of prostaglandins. The reaction catalysed by d-6-d (delta-6-desaturase) is the rate limiting step in the metabolism of EFAs. DGLA can also be converted to AA by the action of the enzyme delta-5-desaturase. AA forms the precursor of 2 series of prostaglandins, thromboxanes and 4 series leukotrienes.

ALA is convetred to EPA by d-6-d- and d-5-d. EPA forms the precursor of 3 series of prostaglandins and 5 series of leukotrienes. The activity of d-6-d and d-5-d are genetically determined. Both d-6-d and d-5-d are present in almost all tissues of the body. The conversion of EFAs to prostaglandins and thromboxanes occurs via the cyclo-oxygenase pathway where as the formation of leukotrienes is by the lipoxygenase pathway. Now it is known that EFAs and their metabolites can bring about a host of actions which are

extremely important and are of clinical significance even though they form precursors to the formation of PGs, TXs (thromboxanes) and LTs (leukotrienes) which also have important biological actions. LA, GLA, DGLA, AA, ALA, EPA and DHA are all polyunsaturated fatty acids and only LA and ALA are essential fatty acids (EFAs). Hence, when the term EFAs is used it refers to LA and ALA where as when the term PUFAs is used it refers to LA, GLA, DGLA, AA, ALA, EPA and DHA.

U.S. patent 5,763,484 to Horrobin, issued on June 9, 1998 teaches a method of treatment of cancer using one or more metabolites of linoleic acid, and one or more metabolites of alpha-linolenic acid, administered in the form of an ester, salt, amide or other derivative which is convertible in the body. The Horrobin patent also states that human beings with atopy, a condition caused by abnormal immune responses may have a deficiency in the ability to convert linoleic acid to gamma-linolenic acid.

U.S. patent 5,246,726 to Horrobin et al, issued on Sept 21, 1993 teaches a method of treating cancer using iron compounds and essential fatty acids, particularly gamma-linolenic acid, dihomo-gamma-linolenic acid or eicosapentaenoic acid in quantities of 1 mg to 100 gm daily. U.S. patent 5,603,959 to Horrobin et al, issued February 18, 1997 teaches methods of treatment for rheumatoid arthritis, osteoarthritis and other disorders including Alzheimer's disease, using essential fatty acids. In particular, in

this Horrobin et al patent, a non-steroidal anti-inflammatory drug (NSAID) which is chemically linked to an essential fatty acid is used for treatment.

U. S. patent 5,795,909 to Shashoua et al, issued Aug 18, 1998 teaches the use of cis-docosahexaenoic acid and taxanes in treating cell proliferative disorders.

A Jan 15, 1992 publication by Hayashi Y, et al, in Cancer Research, titled "Anticancer effects of free polyunsaturated fatty acids in an oily lympho-graphic agent following intrahepatic arterial administration to a rabbit bearing VX-2 tumor", teaches the antihepatic cancer effects of three free polyunsaturated fatty acids (linoleic, aplha-linolenic and gamma-linolenic acids). The publication states that intrahepatic arterial administration of Lipiodol® containing the free fatty acids is an effective method of delivery of the three fatty acids as anticancer agents.

An October 1990, publication in Chem Pharm Bulletin 38 (10): 2874-6, authored by Hayashi Y, et al, teaches the release characteristics of a free polyunsaturated fatty acid from an oily lymphographic agent. The prolongation of alpha-linolenic acid release from Lipiodol as a requisite for selective anticancer effect is discussed in the publication.

A May-June 1994 publication by Kinoshita et al in In Vivo 8 (3) : 371-4

investigates the effects of purified linoleic acid (LA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on mammary tumor transplanted into mice. Also investigated by the authors Kinoshita et al was the number of lung metastases. A November 1990 publication in J Pharmacobiodyn 13 (11) : 705-11 by Hayashi Y, et al examines the in vitro cytotoxicity of gamma-linolenic acid against rat hepatoma cells. It is reported that the cytotoxicity of gamma-linolenic acid was correlated closely with the concentration of unbound (free) gamma-linolenic acid. Lipid peroxidation was observed to be inhibited by serum albumin. The publication states that the presence of albumin suppresses the cytotoxicity of an administered free fatty acid.

In a 1992 September-October ; 8(5): 343-347 publication in Nutrition by Ramesh G, et al demonstrated that the cytotoxicity of GLA (which is present in evening primrose oil) is prevented by albumin and other proteins present in the ascitic fluid of animals which have ascitic tumor. The albumin is able to bind to GLA and thus, prevent its ability to the tumor cells. This indicated that whenever GLA and other fatty acids, when given orally or parenterally, might bind to albumin of the plasma which in turn would suppress the cytotoxicity of the fatty acids against tumor cells.

A 1990 publication by Dr. U.N. Das in Nutrition Vol 6, No. 6

November/December 1990, pp 429, described the cytotoxic action of lymphokines, anticancer drugs and c-UFAs. The publication lists abnormal metabolic events in most tumor cells to include:

(i) decreased free radical generation coupled with a relative increase in antioxidant capacity,

(ii) decrease in the content of polyunsaturated fatty acids (PUFAs) which are necessary to trigger oxidative metabolism in human neutrophils and tumor cells,

(iii) an increase in polyamines and

(iv) genetic abnormalities which may partly be responsible for the growth, invasion and metastasis of tumor cells and the development of resistance to anticancer drugs.

Lymphokines, eg., in the form of interferon and tumor necrosis factor, the publication observed, seems to be antitumorous by releasing c-UFAs from the cell membrane lipid pool and consequently inducing free radical generation. Also, drugs such as vincristine augment free radical generation and lipid peroxidation.

A 1991 publication by M.R.C. Naidu, U.N. Das and A. Kishan in "Prostaglandins Leukotrienes and Essential Fatty Acids" 1992 45, (181-

184), discusses selective delivery of gamma-linolenic acid (GLA) to the tumor cells, and intratumoral injections of GLA in the management of gliomas.

A 1993 publication by Sangeetha Sagar and U.N. Das in Med. Science. Research 1993; 21: 457-459 suggests that both anticancer drugs and cUFAs can augment free radical generation and lipid peroxidation.

A 1991 publication by Dr. U.N. Das, in Cancer Letters, 56 (1991) 235-243 (Elsevier Scientific Publishers, Ireland Ltd), states that vitamin E, uric acid, glutathione peroxidase, superoxide dismutase and adenosine triphosphate (ATP) can block, where as iron and copper enhance the tumoricidal action of GLA.

A 1998 publication authored by U.N. Das and others, in Prostaglandins Leukotrienes and Essential Fatty Acids 1998, 58 (1) 39-54 states that cell cycle proteins called cyclins have a crucial role in controlling cell proliferation. The publication also teaches that as experiments indicate, preincubation of vincristine resistant cells with doses of cis-unsaturated fatty acids enhances the cytotoxic action of vincristine.

Summary of the Invention:

The invention in one aspect teaches a method of interrupting blood supply

to a tumor region causing necrosis with very desirable results. The invention also provides a method of causing anti-angiogenic action in the tumor region with the result that new blood vessels and collaterals are not formed to sustain the tumor in the tumor region treated according to the invention.

The present invention in another aspect tackles the issue of drug delivery to the target tissue and provides the most efficacious method of administering an admixture of selected PUFAs along with other elements as will be described hereinafter.

Tumor cells are deficient in phospholipase A2, an enzyme necessary for the release of various PUFAs from the cell membrane lipids as a result of which the production of anti-neoplastic PGs such as PGD2 are not elaborated. In addition, tumor cells secrete an excess of PGE2, an immunosuppressive and mutagenic substance. Further, tumor cells are deficient in PUFAs such as GLA, AA, EPA and DHA due to the low activity of delta -6-desaturase. As a result of these metabolic changes, tumor cells are able to effectively circumvent body's defense and survive. The present invention provides a method of causing necrosis of tymor cells despite their known survival pattern.

Anti-cancer actions of PUFAs:

Tumor cells are not only deficient in PUFAs but also have low rate(s) of

lipid peroxidation, contain relatively large amounts of antioxidants such as vitamin E and superoxide dismutase (SOD). It is also believed that low rates of lipid peroxidation and consequent low amounts of lipid peroxides in the cells can contribute to an increase in the mitotic process which ultimately leads to an increase in cell proliferation. Thus, a deficiency of PUFAs, high amounts of antioxidants and the presence of low amounts of lipid peroxides in the tumor cells can contribute to the growth of tumor cells. This is supported by studies by the inventor wherein it was noted that PUFAs such as GLA, DGLA, AA, EPA and DHA can decrease tumor cell proliferation. In addition, it was also observed that when appropriate amounts of GLA, DGLA, AA, EPA and DHA were added to tumor cells and normal cells, obtained from American Type Culture Collection, only tumor cells were killed without having any significant action on the survival of normal cells in vitro. In mixed culture experiments, in which both normal and tumor cells were grown together, GLA showed more selective tumoricidal action compared to AA, EPA and DHA though, these latter fatty acids were also effective to some extent. This indicated that selective delivery of GLA, DGLA, AA, EPA and DHA to tumor cells may offer a new therapeutic approach in the treatment of cancer.

These in vitro results are supported by in vivo studies performed in animal tumor models. For example, it was noted that GLA, DGLA, AA, EPA and

DHA when used either in the form of pure fatty acid alone or in the form of fatty acid rich oils could inhibit the growth of skin papilloma in mice, formation and growth of hepatoma in rats and ascitic tumor cells in the peritoneum of experimental animals. These results indicate that these fatty acids can inhibit the growth of a variety of tumors even in vivo. In further studies, it was noted that these fatty acids are able to enhance free radical generation and the lipid peroxidation process selectively in the tumor cells but not so much in the normal cells and thus, are able to bring about their cancer killing action.

This ability of PUFAs to augment free radical generation and lipid peroxidation in the tumor cells is analogous to the anti-tumor action of lymphokines such as tumor necrosis factor (TNF) and interferon (IFN), both alpha and gamma varieties. These lymphokines (also referred to as cytokines) are capable of inducing the release of PUFAs from the cell membrane lipid pool and enhance free radical generation in the cells. Similarly several anti-cancer drugs such as, but not limited to, doxorubicin and vincristine have the capacity to augment free radical generation and promote lipid peroxidation. In addition, PUFAs and their products can modulate immune response, augment a respiratory burst of neutrophils and free radical generation by macrophages. This evidence is further testified by the observation that the incidence of cancer in Eskimos is low as influenced by their traditional diet, which is rich in EPA and DHA.

Inventor's studies have shown that PUFAs can be exploited as possible anti-cancer agents either alone or in combination with lymphokines and traditional anti-cancer drugs.

In a series of investigations by the inventor, it was also observed that the cytotoxic action of anti-cancer drugs such as doxorubicin, vincristine and cis-platinum can be augmented by various PUFAs such as GLA, DGLA, AA, EPA and DHA. In addition, these fatty acids could also enhance the cellular uptake of these anti-cancer drugs by the tumor cells and thus, are able to potentiate the anti-cancer actions of these drugs. In another similar experiment by the inventor, it was also observed that GLA, DGLA, AA, EPA and DHA were able to kill TNF resistant L-929 tumor cells in vitro. Further, these TNF-resistant tumor cells were made TNF sensitive by prior treatment of these L-929 cells by GLA, DGLA, AA, EPA and DHA. These results indicated that PUFAs can not only kill the tumor cells by themselves but are also capable of potentiating the cell killing effect of various anti-cancer drugs, lymphokines such as TNF and IFN and also render anti-cancer drug and TNF-resistant tumor cells sensitive to the cytotoxic action of various anti-cancer drugs and lymphokines.

In another set of experiments, it was also noted that vincristine resistant tumor cells, KB-^{chR} 8-5 (henceforth referred to as KB-8-5 cells) can be made sensitive to the cytotoxic action of vincristine by GLA, DGLA, AA, EPA

and DHA. Further, when sub-optimal doses of vincristine and fatty acids were added together to these vincristine resistant cells produced optimal (i.e. significant) cell killing action. This suggests that vincristine and other anti-cancer compounds and PUFAs when added together to cancer cells, they potentiate the cytotoxic action of each other. Fatty acid analysis of both vincristine sensitive (KB-3-1) and resistant (KB-8-5) cells revealed that the resistant cells have low amounts of GLA, AA, EPA and DHA compared to the vincristine sensitive tumor cells indicating that a deficiency of these fatty acids may be responsible for their resistance to the cytotoxic actions of anti-cancer drugs. Since, both vincristine sensitive and resistant tumor cells are easily (and to the same extent) killed by various PUFAs in vitro, this suggests that even drug-resistant tumor cells can be killed by these fatty acids.

In yet another set of experiments, the inventor also noted that L-929 cells which are resistant to the cytotoxic action of tumor necrosis factor (referred to as TNF-resistant L-929 cells) can also be made sensitive to the cytotoxic action of TNF by pre-treating these cells with various PUFAs. In other words, L-929 cells which are resistant to the cytotoxic action of TNF can be sensitized to the cytotoxic action of TNF by PUFAs. This again indicates that PUFAs can not only kill the tumor cells but can also serve as sensitizing agents rendering various tumor cells responsive to the cytotoxic action of various anti-cancer drugs and lymphokines (cytokines) such as tumor

necrosis factor.

Human studies:

Though many in vitro and animal studies suggested that a variety of compounds can kill tumor cells, it is not always possible to extrapolate these studies to the human situation. Many compounds which show excellent cancer killing effect in the laboratory may not hold any promise when they are used in the humans. In order to verify the possible use of various PUFAs in humans, the inventor performed clinical studies in patients with Hodgkin's and non-Hodgkin's lymphoma, human brain gliomas, primary hepatoma (liver cancer) and giant cell tumor of the bone. In all these studies, it was observed that these PUFAs, in particular GLA, could not only arrest the growth of the tumor but also regress the size of the tumor to a significant degree. In the case of Hodgkin's and non-Hodgkin's cancer the PUFAs, GLA, was given orally, whereas in the case of human brain glioma (malignant gliomas and other malignant tumors of the brain) GLA was given as an intra-tumoral injection into the tumor bed. In the case of hepatoma and giant cell tumor of the bone GLA in the inventor's study was given in combination with an oily lymphographic agent as an emulsion by selective intra-arterial catheterization. In all these studies no significant side effects were noted and all the patients responded to the treatment. Of significance in this context is the fact that PUFAs can bind to albumin and other proteins

and hence, if given intravenously may not be available to be taken up by the tumor cells and consequently may not be able to bring about their cell killing action on the tumor cells. In view of this, it is essential that PUFAs including GLA should be delivered to the patients in such a manner that it is easily available to the tumor (tumor cells) and is delivered selectively to the tumor cells. It is highly desirable that PUFAs including GLA be given intra-tumorally as was experimentally done in the case of human gliomas, or, intra-arterially by selective intra-arterial infusion as was done experimentally in the case of hepatoma and giant cell tumor of the bone. But, it is also possible that in some cases of cancer such as Hodgkin's and non-Hodgkin's lymphoma wherein the tumor cells are extremely sensitive to the cytotoxic actions of PUFAs, even oral administration may be sufficient as was observed in certain patients. Since, PUFAs can potentiate the cell killing effect of anti-cancer drugs and lymphokines, it is desirable to administer a combination of PUFAs, anti-cancer drugs, lymphokines such as TNF and interferon or a combination thereof with or without a carrier agent such as an oily lymphographic agent as the situation indicates. Further studies have also revealed that PUFAs such as GLA, DGLA and EPA can prevent or ameliorate the side effects of anti-cancer agents such as gamma-radiation and cis-platinum to the bone marrow cells of mice. Thus, it appears that when PUFAs and conventional anti-cancer drugs/agents are given together they not only potentiate the cytotoxic action of each on the tumor cells and thus, produce a synergistic and/or additive action in their ability to

eliminate the tumor cells but it will also lead to elimination, reduction or amelioration of the side effects of conventional anti-cancer agents. Since PUFAs are able to potentiate the cytotoxic acton(s) of conventional anti-cancer agents and lymphokines, it is also possible that this will lead to a significant reduction in the doses of these latter agents without compromising the ultimate benefit namely, elimination of tumor cells or the tumor.

Some of the phenomena which reduce the efiicacy of the cytotoxic action of PUFAs and conventional anti-cancer drugs/agents in vivo as compared to in vitro results include the following:

a. PUFAs when administered orally or intravenously can bind to albumin and other proteins in living beings and may not be available to be taken up by the tumor cells.

b. The cytotoxic action of PUFAs is produced by the augmentation of free radical generation and lipid peroxidation in only tumor cells (but not in normal cells). The intensity of the cytotoxic action is disadvantageously reduced in actual clinical efforts because of inefficient transportation of the fatty acids to the target areas.

c. Continued blood supply to tissue with proliferative cell disorders is not conducive to bringing about a significant amount of necrosis especially if the malignant cells multiply faster than they are being destroyed.

d. It was found from a study reported in a June, 1994 "Cancer letters" publication authored by N. Madhavi and U.N. Das that antioxidants like vitamin E and the superoxide anion quencher, superoxide dismutase (SOD) could completely inhibit radical generation and lipid peroxidation generated by PUFAs like GLA, EPA and DHA. It appears that selective drug delivery to the target tissue will be conducive to the efficacy of the beneficial action of the PUFAs.

The invention in one aspect resides in a method of inhibiting blood supply to a tumor, comprising the steps of: locating an artery which carries major blood supply to the tumor, said artery being one that is proximate to the tumor, and intra-arterially injecting into the located artery a predetermined quantity of a polyunsaturated fatty acid (PUFA) in the form of a solution of at least one PUFA chosen from LA, GLA, DGLA, AA, ALA, EPA and DHA.

The invention in another aspect resides in a method for treating tumors and for facilitating visualization of remission of the tumor responsive to treatment, comprising the steps of (a) locating an artery which carries a major portion of blood supply to the tumor and is adjacent to the tumor; (b) obtaining an initial radiographic image of the tumor region; (c) injecting into the artery a mixture of (i) an oily lymphographic agent, (ii) a lithium salt solution of at least one PUFA chosen from LA, GLA, DGLA, AA, ALA, EPA and DHA;

(d) obtaining second and subsequent radiographic images of the tumor regions after predetermined lapses of time; and comparing the initial radiographic images with the second and subsequent radiographic images to assess the extent of remission of the tumor.

The invention in another aspect resides in a method of causing necrosis in a cancerous tumor by inhibiting blood supply to the tumor, comprising the steps of :

(a) locating an artery proximate to the tumor which carries major blood supply to the tumor;

(b) injecting into the located artery a mixture of (i) an oily lymphographic agent; (ii) a lithium salt solution of at least one essential fatty acid chosen from LA,GLA, DGLA, AA, ALA, EPA and DHA

(c) waiting for a predetermined time period and assessing a degree of necrosis in the tumor by examining by a radiographic study or by other means; and

(d) repeating step (b) if necessary to increase the necrosis.

In yet another aspect, the invention resides in a method of treating a glioma and visualizing remission of the glioma as it responds to treatment, comprising :

(a) obtaining an initial radiographic image of a region containing the glioma;

(b) injecting into the glioma region an admixture of (i) a sodium salt or any other suitable salt solution of at least one polyunsaturated fatty acid chosen from LA, GLA, DGLA, AA, ALA, EPA and DHA or a combination there of;

(c) obtaining second and subsequent radiographic images of the glioma region after predetermined lapses of time; and comparing the initial radiographic pictures which shows the glioma , with second and subsequent radiographic images of the glioma region to visualize and assess the extent of remission of the glioma.

In yet another aspect, the invention resides in a method of treating mammalian cell proliferative disorders using an emulsion of a lithium salt of a PUFA or combinations of PUFAs and a predetermined anticancer drug, administered parenterally. Preferably, the intra-arterial administration of the admixture containing PUFA(s) is done through a catheter. Also, the artery carrying major blood supply to the tumor is to be understood herein as synonymous to the artery which will supply the tumor feeding vessels. Owing to a phenomenon which is consequent to inhibiting blood supply, the present invention makes it not conducive to the formation of new blood vessels i.e. angiogenesis.

Brief description of the illustrations

A more detailed understanding of the invention may be had from the following description of preferred embodiments, given by way of example, and to be understood in conjunction with the accompanying illustrations/drawings wherein:

Figure 1 illustrates the structural metabolism of essential fatty acids;

Figures 2 and 3 illustrate radiographic images of the giant cell tumor of the human scapula before and after receiving treatment as per a preferred method of the invention;

Figures 4 and 5 illustrate radiographic images of hepatoma of a human patient before and after receiving treatment as per a preferred method of the invention;

Figures 6 and 7 illustrate images of a giant cell tumor of the lower end of the femur (close to the knee joint area) before and after treatment using the invention; and

Figures 8 to 13 illustrate sequential CAT scan images of the abdomen of a human patient with hepatoma in the course of the treatment using the present invention.

Detailed description of embodiments

Figure 1 shows a typical known metabolism pattern of essential fatty acids as known in prior art. Essential fatty acids are precursors of eicosanoids and are important structural components of cell membranes. They also provide the substrates for the generation of lipid peroxidation products which have an inhibitory action on cell proliferation. Tumor cells are known to have low delta-6-desaturase activity, an enzyme necessary for the desaturation of dietary linoleic acid (LA, 18:2, n-6) and alpha-linolenic acid (ALA, 18:3, n-3) to their respective products. In an earlier study, the inventor has shown that hepatocarcinogens, diethylnitrosamine (DEN) and 2-acetylamino-fluorine (2-AAF), can suppress the activity of delta-6-desaturase and delta-5-desaturase resulting in low levels of gamma-linolenic acid (GLA, 18:3, n-6) and arachidonic acid (AA, 20:4, n-6) and eicosapentaenoic acid (EPA, 20:5, n-3) and docosahexaenoic acid (DHA, 22:6, n-3) in the tumor cells. These results led the inventor and others to study the effect of various fatty acids on the survival of tumor cells in vitro. Addition of EFAs (LA and ALA) and other PUFAs such as GLA, DGLA, AA, EPA and DHA to a variety of tumor cells in vitro showed that only tumor cells are killed by these fatty acids without harming the normal cells. This selective tumoricidal action of fatty acids seems to be mediated by free radicals and lipid peroxides. Similar to these fatty acids, radiation, some anti-cancer drugs and cytokines (lymphokines) also seem to have the ability to generate free

radicals in tumor cells and thus, bring about their tumoricidal actions.

Since drug resistance is a major obstacle in the clinical treatment of cancer and as PUFAs have selective tumoricidal action, the inventor studied the effects of PUFAs on drug-resistant tumor cells and their modulating influence on the actions of anti-cancer drugs.

In the above context, in addition to producing reversal of tumor cell drug resistance by the administration of polyunsaturated fatty acids, it is seen in the invention that the manner of targeting the cancerous tissue is very critical to the efficacy and the speed with which necrosis can be brought about. More particularly, it is realized through this invention that by delivering a chosen admixture of salts of predetermined polyunsaturated fatty acids and predetermined anti-cancer agent(s) to the tumor site intra-arterially by using the artery most proximate to the tumor site, a very beneficial and hitherto unknown effect in terms of inhibiting blood supply to the tumor site is achieved.

In clinical studies conducted by the inventor, the inhibition of blood supply was pronounced enough to cause cutting off blood supply to the tumor site with very little time lag. In other instances, an unmistaken strangling of blood supply to the tumor region was observed, but was relatively gradual.

In the following description of observation and clinical study regarding the treatment of patients with hepatoma, giant cell tumor of scapula and giant cell tumor of the femur, the unexpected beneficial effect of inhibition of blood supply to the tumor region caused by intra-arterial injection of a mixture comprising PUFA salts injected into an artery close to the tumor region and normally supplying the tumor with its major blood supply can be visualized.

Figure 2 shows an actual radiographic image of a giant cell tumor of scapula of a human patient. The tumor is shown at 21 in figure 2. The major portion of the blood supply to the tumor happens to be through the subclavian artery which is shown at 22. The subclavian artery continues as axillary artery and later it continues as brachial artery to supply blood to the upper arm. In this instance since the subclavian artery 22 happens to be the major provider of blood supply to the tumor 21, intra-arterial injection of the admixture of selected PUFA salts, and an oily lymphographic agent was made into the subclavian artery 22 through catheter 23. Figure 3 shows the actual radiographic image of the tumor shown in figure 2, after a very little time lapse, wherein the blood supply inhibition can be observed. In fact, the inventor in clinical studies noted such an immediate response after an intra-arterial injection that because of the quick blockage of the blood path, no further administration of the admixture was easily possible or necessary.

Figure 4 shows a radiographic image of a hepatoma which is a tumor of

the liver. Intra-arterial administration of of an admixture of the lymphographic agent and selected PUFA salts was done with the use of a catheter inserted into the hepatic artery 42, via the coeliac axis which was determined the provider of the major blood supply to the afflicted region. Coeliac axis, as known, is a major junction of the abdominal aorta from which major blood vessels emanate including the hepatic artery to supply the liver. The radiographic image of figure 5 was taken after a time lapse of four weeks. From the image in figure 5, it may be seen that the lymphographic agent as distributed is visible in the tumor region, with the blood supply into the tumor region being on the decline. As described earlier, the exposure of healthy cells and tissue to PUFAs or its salt derivatives in any form, does not adversely influence the condition or integrity of healthy tissue. The blood supply, for an unknown reason gradually diminishes to the point of being totally cut off, after intra-arterial administration of a mixture of selected PUFAs salts and optional predetermined anti-cancer drugs in an oily lymphographic agent. In fact, experiments revealed that blood supply was cut off with the intra-arterial administration of PUFAs only, without the addition of any anti-cancer drugs. The presence of an oily lymphographic agent as a carrier renders the distribution and retention of the lymphographic agent visible which demonstrates that PUFA salts and anti-cancer drugs indeed reached the target tissue through the carrier. The undesirable alternative to accomplishing massive necrosis of the cells in the tumor region will be removal of the malignant

liver by surgery which is extremely difficult and hazardous and this can sometimes result in torrential bleeding during surgery or following surgery as these tumors are usually highly vascular which would ultimately result in the patient's death.

Figures 6 and 7 show radiographic images of a giant cell tumor 61 of the bone in the human femur close to the knee joint area, which is known as the Popliteal fossa. Popliteal artery 62, vein (not shown), and nerve (not shown) traverse the Popliteal fossa region. If the tumor of the bone in the Popliteal fossa region were to be physically removed by excision, it would be a mutilating surgery to the patient. Using the method of the preferred embodiment of this invention, blood supply to the Popliteal fossa region was inhibited to the point of killing the tumor cells, where upon the bone has an opportunity to regrow. In the case of the illustration in figures 6 and 7, a preferred embodiment of the present inventive method was used to inhibit blood supply to the tumor region and bring about massive necrosis in the tumor region, giving an opportunity for healthy bone growth to be recovered.

Figures 8 to 13 illustrate radiographic images of sequential computer axial tomography scans of a patient abdomen without contrast showing hepatoma during the course of treatment using the present invention.

Figure 8 shows the afflicted region i.e. the liver marked before

administering an admixture which was prepared according to a preferred embodiment of the invention. The following table shows the time lapses after which the images of the several figures were taken:

Figure 8 - day 1 before administering admixture

Figure 9 - day 1 after injecting admixture

Figure 10 - day 2 after injecting admixture

Figure 11 - day 4 after injecting admixture

Figure 12 - 3 ½ weeks after injecting admixture

Figure 13 - 4 ½ weeks after injecting admixture.

Figure 9 shows CAT scan image almost immediately after administering the admixture. Figure 10 shows a CAT scan image taken less than a day after the images of figures 8 and 9. It is seen from figure 10 that the oily lymphographic agent is visibly distributed and is seen as a whitish material. In figure 11 the response to the treatment can be observed. In figures 12 and 13, the images illustrate remission, and the return of the liver tissue to normalcy in about 27 days after the treatment began. Even though the examples of patient treatment given above, described in conjunction with illustrations in figures 2 to 13 relate generally to intra-arterially injecting an admixture of a predetermined salt of PUFA combined with a lymphographic agent/carrier (with or without a selected anti-cancer agent/drug), the inventor found promising results even in situations wherein the admixture was taken in a non-parenteral manner, eg., as capsules. As will be described in more

detail herein after, a combination of two or more methods of taking the admixture, i.e., oral and parenteral showed very encouraging results.

One aspect of the invention consists in the preparation of a combination/composition of treatment of cancer in which one or more of LA, GLA, DGLA, AA, ALA, EPA and DHA are administered with conventional anti-cancer agents/drugs including lymphokines such as TNF and interferon with or without in an oily lymphographic agent or any other suitable agent for the delivery these compounds; optionally, radiation may be included. The PUFAs may be provided in a daily dose of 0.5 mg to 50 gm together with appropriate doses of conventional anti-cancer drugs such as vincristine, doxorubicin, L-asparaginase, cis-platinum, busulfan etc., in a daily/weekly/monthly dose of 1 mg to 50 gm depending on the requirement and the stage of the disease and as may be determined from time to time with or without the addition of lymphokines such as TNF (alpha or gamma variety) and/or interferon (alpha or gamma type) in a dose of 1 ug to 100 mg (in the case of TNF it may be from 1000 units to 10 million units) per day. The combination of PUFAs, conventional anti-cancer drugs, lymphokines and the oily lymphographic agent may be administered by any one or different routes at the same time or at different times and intervals by selecting an appropriate route for each administration or in combination eg. oral, parenteral including intra-arterial infusion, topical, anal, vaginal routes as suppositories or local injection direct into the tumor

bed under the guidance of appropriate equipment such as but not limited to radiological guidance (X-rays), CT guidance or MRI guidance or by stereostaxic guidance. The daily dose(s) of these compounds may not exclude the administration of long acting preparations or depot preparation once or more times in a day, week, month or at some other appropriate time interval as determined from time to time depending on the necessity. The fatty acids (PUFAs) may be present in any physiologically acceptable form including but not limited to glycerides, esters, free acids, amides, phospholipids or salts. The conventional anti-cancer drugs may be administered by themselves or in conjugation with PUFAs (either alone or in combination such as GLA alone or GLA + AA, LA, DGLA, ALA, EPA or DHA). Similarly lymphokines such as TNF and IFN may be given by themselves or in conjugation with PUFAs. For intra-arterial infusion or administration of LA, GLA, DGLA, AA, ALA, EPA and/or DHA these may be given by themselves or in combination or dissolved or conjugated in/with the oily lymphographic agent or any other suitable solution that can be given parenterally but not limited to them. All these PUFAs, conventional anti-cancer drugs, lymphokines and lymphographic agent may be given each alone or in combination thereof or all together or separately at the same time or at different time intervals on the same day/week/month either by same route or different routes as the situation demands.

Examples:

1. Hard (wherein the PUFAs have been microencapsulated) or soft gelatin capsules (wherein the fatty acids are present in an oily form) made by accepted norma or forms or methods and are administered to persons suffering from cancer in conjunction with conventional anti-cancer drugs or lymphokines in the doses as stated supra.

2. Hard or soft gelatin capsules made by conventional methods, in which the fatty acids and the anti-cancer drugs are incorporated together in the same capsule and are administered to persons suffering from cancer.

3. As intra-tumoral preparation in appropriate doses (from 0.5 mg to 50 mg per day) of pure LA, GLA, DGLA, AA, ALA, EPA and DHA either individually or in combination thereof especially for the treatment of human brain gliomas or any other accessible tumor (eg. urinary bladder cancer, carcinoma of the esophagus, carcinoma of the lung, breast cancer etc.) by any route by using flexible fiber optic scopes such as bronchoscope, urethroscope, hysteroscope, etc. In the case of tumors of the head and neck the fatty acids are administered either by direct intra-tumoral route or by selective catheterization of the tumor feeding vessel(s) either by femoral, brachial or carotid routes. The PUFAs can be given to these patients daily, weekly or monthly or as and when necessary depending on the requirement and response of the patient to the treatment.

4. Administered as selective intra-arterial infusion or injection into the tumor feeding vessel by femoral, brachial or carotid routes or any other suitable route or in a combination thereof the PUFAs either alone or in combination with anti-cancer drugs/lymphokines with or without the oily lymphographic agent or any other suitable agent all in a mixture or in conjugated form(s) (like GLA + any conventional anti-cancer drug or drugs + lymphokines such as TNF and/or interferon., LA/GLA/DGLA-/AA/ALA/EPA/DHA all individually or in combination thereof + conventional anti-cancer drug(s) + lymphokines such as TNF and/or IFN + lymphographic agent., LA/GLA/DGLA/AA/ALA/EPA/DHA in combination with or conjugated to or emulsified with or mixed with oily lymphographic agent., LA/GLA/DGLA/AA/ALA/EPA/DHA alone or in combination thereof in oily lymphographic agent as a mixture or emulsion or as a conjugate(s) and a variety of other combinations thereof). This preparation may be administered daily, weekly or monthly or at some other appropriate time interval.

5. Topical preparation of PUFAs either alone or in combination thereof with conventional anti-cancer drugs or lymphokines such as TNF/IFN or any other suitable lymphokine in a suitable delivery vehicle in which daily doses (ranging from 0.5 ug to 100 mg) are applied to primary skin cancers including Kaposi's sarcoma locally and/or conventional anti-cancer drugs and/or lymphokines are given either orally or parenterally.

By the different embodiments of the invention method described supra,

(i) PUFAs or cis-EFAs (essential fatty acids described here are also called as cis-fatty acids as by virtue of their structure are referred to as cis-EFAs as they are in cis-configuration) when admnistered to patients intra-arterially or even otherwise as a combination of intra-arterial and oral administration, there are less chances of albumin and other protiens binding to the fatty acids. Consequently, PUFAs thus administered using the invention are better available to be taken up by the tumor cells.

(ii) Owing to the efficient transportation of PUFAs to the tumor site as described hereinbefore, there is increased intensity of the cytotoxic action of PUFAs and the administered anti-cancer agents (drugs or cytokines or a combination thereof). Thus, using the invention, there is relatively better augmentation of free radical generation and lipid peroxidation in the tumor cells, thereby facilitating a greater degree of necrosis.

(iii) Inhibiting blood supply to the tumor region by the method of the invention prevents cell proliferation in the tumor region, thus enabling healthy tissue to grow back into place.

(iv) The inhibition otherwise caused by vitamin E and superoxide dismutase to radical generation and lipid peroxidation produced by

PUFAs, is reduced in the method of this invention because of the manner of transportation of PUFAs to the tumor site intra-arterially through a proximate artery.

It is also within the purview of this invention, as stated supra to administer an admixture of PUFAs, anti-cancer drugs, and selected cytokines intra-arterially, at the same time, administering predetermined doses of PUFAs orally. All such variations are envisaged to be within the ambit of this invention.

Application to mammals: Even though the examples described supra relate to humans, it is envisaged that the method of inhibiting blood supply and admixture of this invention are equally applicable to other mammals.

Equivalents

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims. For example, sodium and potassium salts are considered equivalents of each other. Imaging techniques referred to herein are intended to include CAT, MRI, X-rays and other possible imaging methods. Those skilled in the art will recognize or be able to

ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the appended claims.

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Claims

1. A method of inhibiting blood supply to a tumor, comprising the steps of:

(a) locating an artery which carries major blood supply to the tumor, said artery being one that is proximate to the tumor ; and

(b) intra-arterially injecting into the located artery a predetermined quantity of a polyunsaturated fatty acid in the form of a solution of at least one polyunsaturated fatty acid chosen from linoleic acid, gamma-linolenic acid, dihomo-gamma-linolenic acid, arachidonic acid, alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid.

2. A method as in claim 1 including the step of causing antiangiogenic action, wherein polyunsaturated fatty acid is in the form of a lithium salt solution and wherein said predetermined quantity of the fatty acid is generally in a range of 0.5 mg to 50 gm.

3. A method as in claim 1 wherein step (b) comprises intra-arterially injecting a predetermined quantity of a polyunsaturated fatty acid in the form of a derivative of a polyunsaturated fatty acid including a predetermined lymphokine, said derivative being chosen from glycerides, esters, free acids, amides, phospholipids and salts.

4. A method as in claim 1 wherein the polyunsaturated fatty acid is in the form of a lithium salt solution of gamma-linolenic acid and

eicosapentaenoic acid/docosahexaenoic acid, including a predetermined quantity of tumor necrosis factor and a predetermined anti-cancer drug.

5. A method of treating a tumor and facilitating visualization of remission of the tumor responsive to treatment, comprising

(a) locating an artery which carries a major portion of blood supply to said tumor and is adjacent to the tumor;

(b) obtaining an initial radiographic image of the tumor region;

(c) injecting into the located artery a mixture of at least

(i) an oily lymphographic agent

(ii) a lithium salt solution of at least one polyunsaturated fatty acid chosen from linoleic acid, gamma-linolenic acid, dihomo-gamma-linolenic acid, arachidonic acid, alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid

(d) obtaining second and subsequent radiographic images of the tumor region after predetermined lapses of time; and

(e) comparing the initial radiographic image with the second and subsequent images to assess an extent of remission of the tumor.

6. A method as in claim 5 wherein step (c) comprises intra-arterially injecting a mixture causing anti-angiogenic action by inhibiting the

blood supply to the tumor, wherein further the oily lymphographic agent acts as a carrier, and the lithium salt solution comprises of predetermined quantities of gamma-linolenic acid, eicosapentaenoic acid and/or docosa-hexaenoic acid.

7. A method of treating a glioma and visualizing remission of the glioma as it responds to treatment, comprising:

(a) obtaining an initial radiographic image of a region containing the glioma;

(b) injecting into the glioma region a sodium salt of at least one polyunsaturated fatty acid chosen from linoleic acid, gamma-linolenic acid, dihomo-gamma-linolenic acid, arachidonic acid, alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid.

(c) obtaining second and subsequent radiographic images which show the glioma region after predetermined lapses of time; and

(d) comparing the initial radiographic image which shows the glioma, with second and subsequent radiographic images of the glioma region to visualize and assess an extent of remission of the glioma.

8. A method as in claim 7 wherein the sodium salt solution comprises a solution of predetermined quantities of sodium salt of at least one polyunsaturated fatty acid .

9. A method of treating a cancerous tumor, comprising

(a) using an oily lymphographic agent as a carrier for

(i) at least one polyunsaturated fatty acid chosen from a lithium salt of at least one of linoleic acid, gamma-linolenic acid, dihomo-gamma-linolenic acid, arachidonic acid, alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid

(ii) one predetermined anti-cancer drug, and

(b) administering a predetermined quantity of selected fatty acids and predetermined anti-cancer drug(s) in the oily lymphographic agent as a carrier.

10. A method of treating a mammalian cell proliferative disorder including cancer with a polyunsaturated fatty acid such as gamma-linolenic acid, arachidonic acid, eicosapentaenoic acid or docosahexaenoic acid or combinations thereof, comprising:

preparing the polyunsaturated fatty acid in the form of a lithium salt in an emulsion with an oily lymphographic agent including covalent conjugation with a pharmaceutical agent chosen from vincristine, adriamycin, doxorubicin, cyclophosphamide, cis-platinum, L-asparaginase, procarbazine, camptothecin, taxol or busulfan; and administering said emulsion parenterally.

11. The method of claim 10 wherein the emulsion includes at least one

biological compound which is naturally occurring in the body and which has an anti-cancer action, said biological compound being chosen from tumor necrosis factor, alpha or gamma-interferon.

12. The method of claim 11 wherein the essential fatty acid metabolite selected is from a lithium salt of gamma-linolenic acid or docosahexaenoic acid in an amount of 0.5 mg to 50 gm.

13. The method of claim 12 wherein the oily lymphographic agent is used as a carrier.

14. The method of claim 10 wherein the disorder is any type of cancer including but not limited to hepatoma, bronchogenic cancer of the lung, colon cancer, breast cancer, ovarian cancer, cancer of the kidney such as hypernephroma, skin cancer such as melanoma, Kaposi's sarcoma, cancer of the esophagus, cancer of the stomach, leukemias of all types or lymphomas of all types and wherein the emulsion includes a predetermined quantity of a lymphokine.

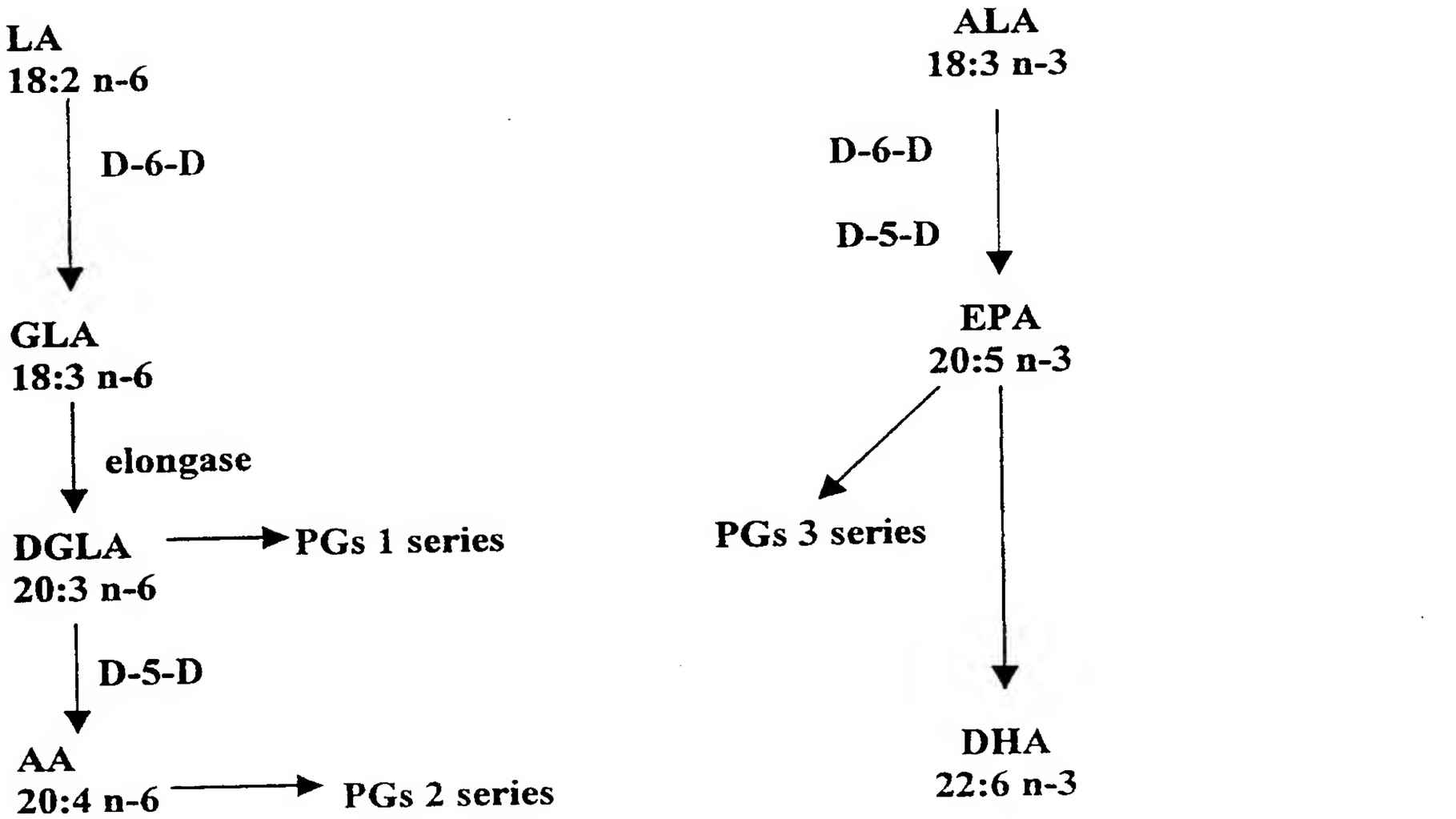

FIGURE 1 SHOWING THE METABOLISM OF ESSENTIAL FATTY ACIDS

PRIOR ART

FIGURE 1

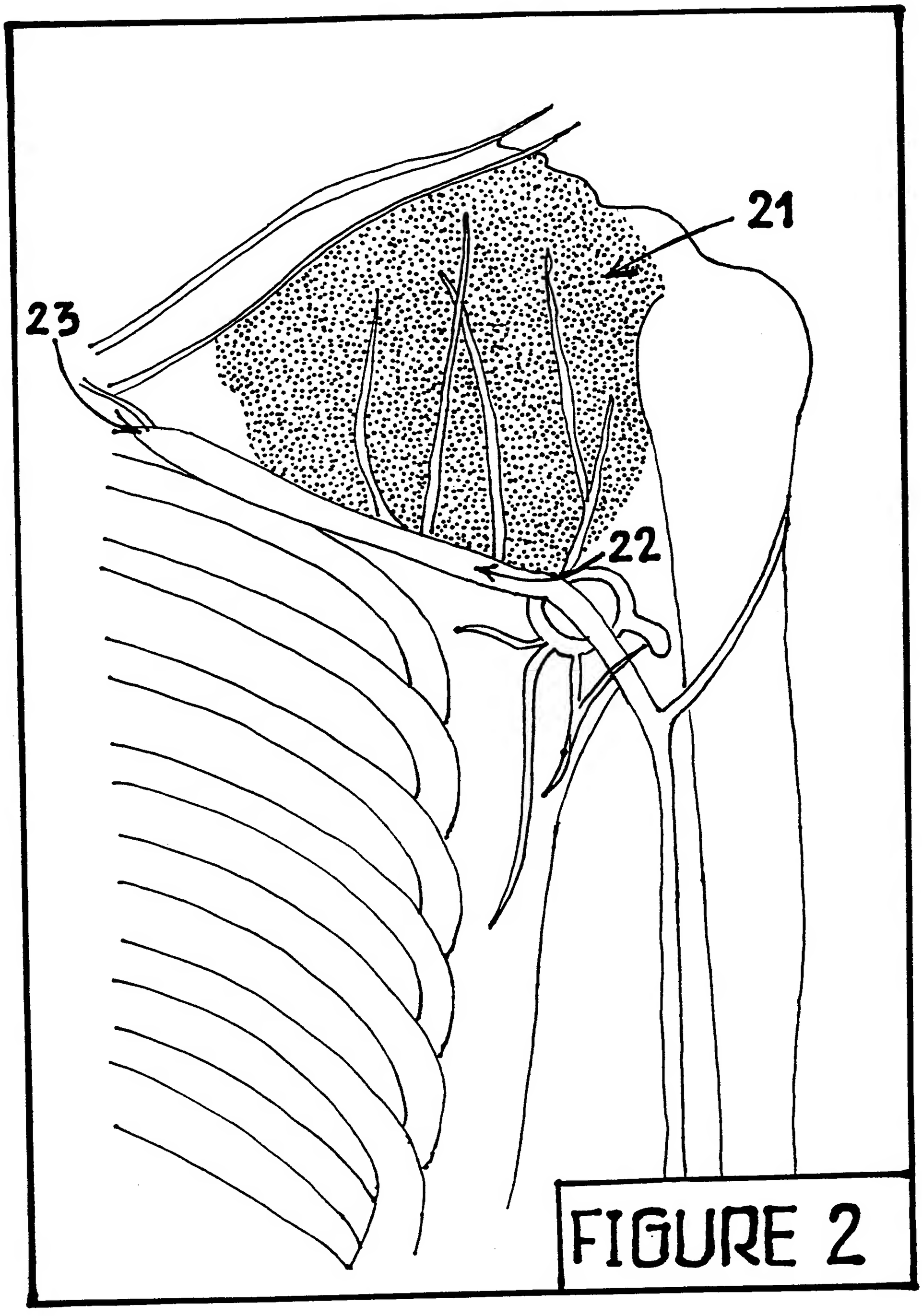

FIGURE 2

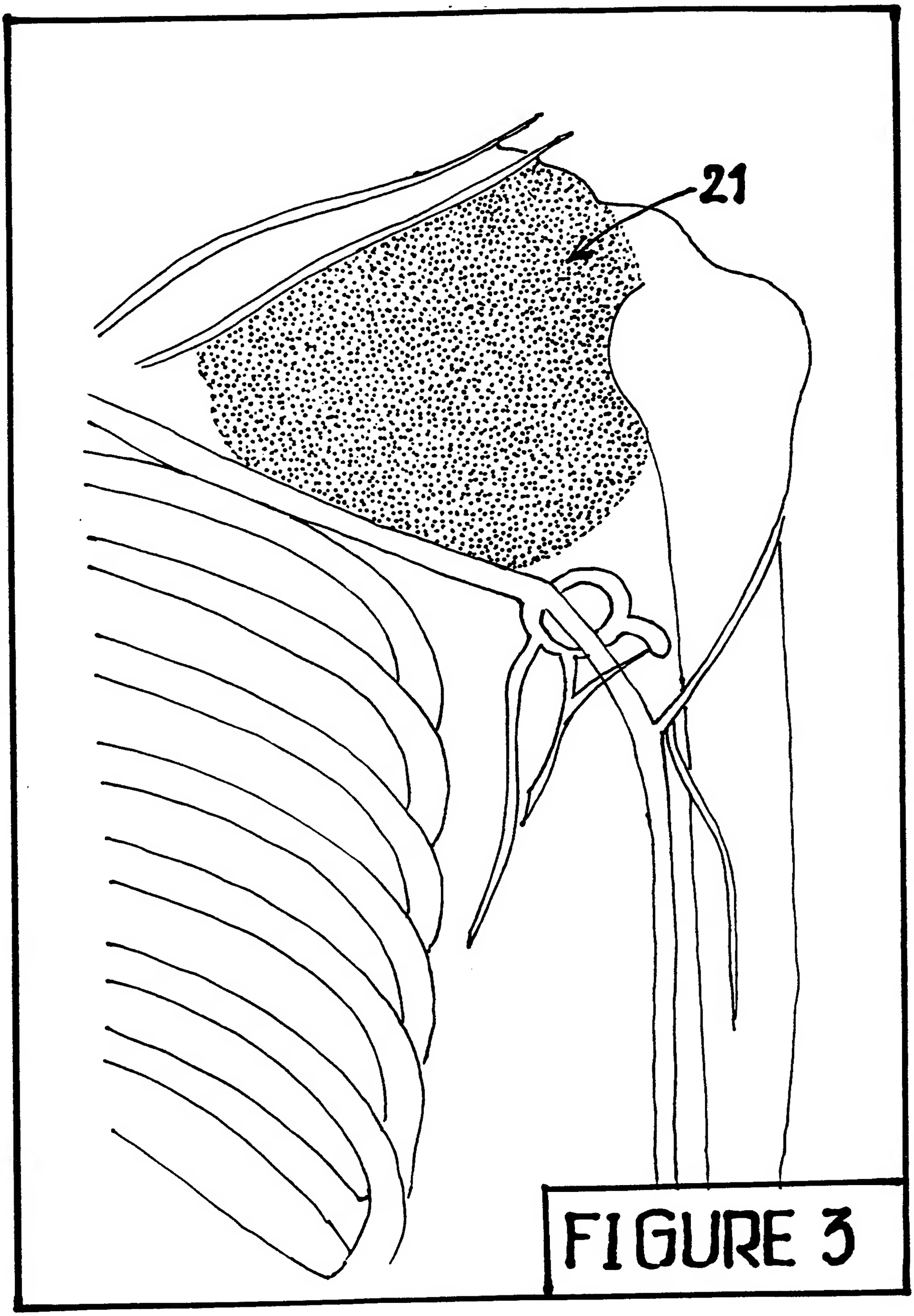

FIGURE 3

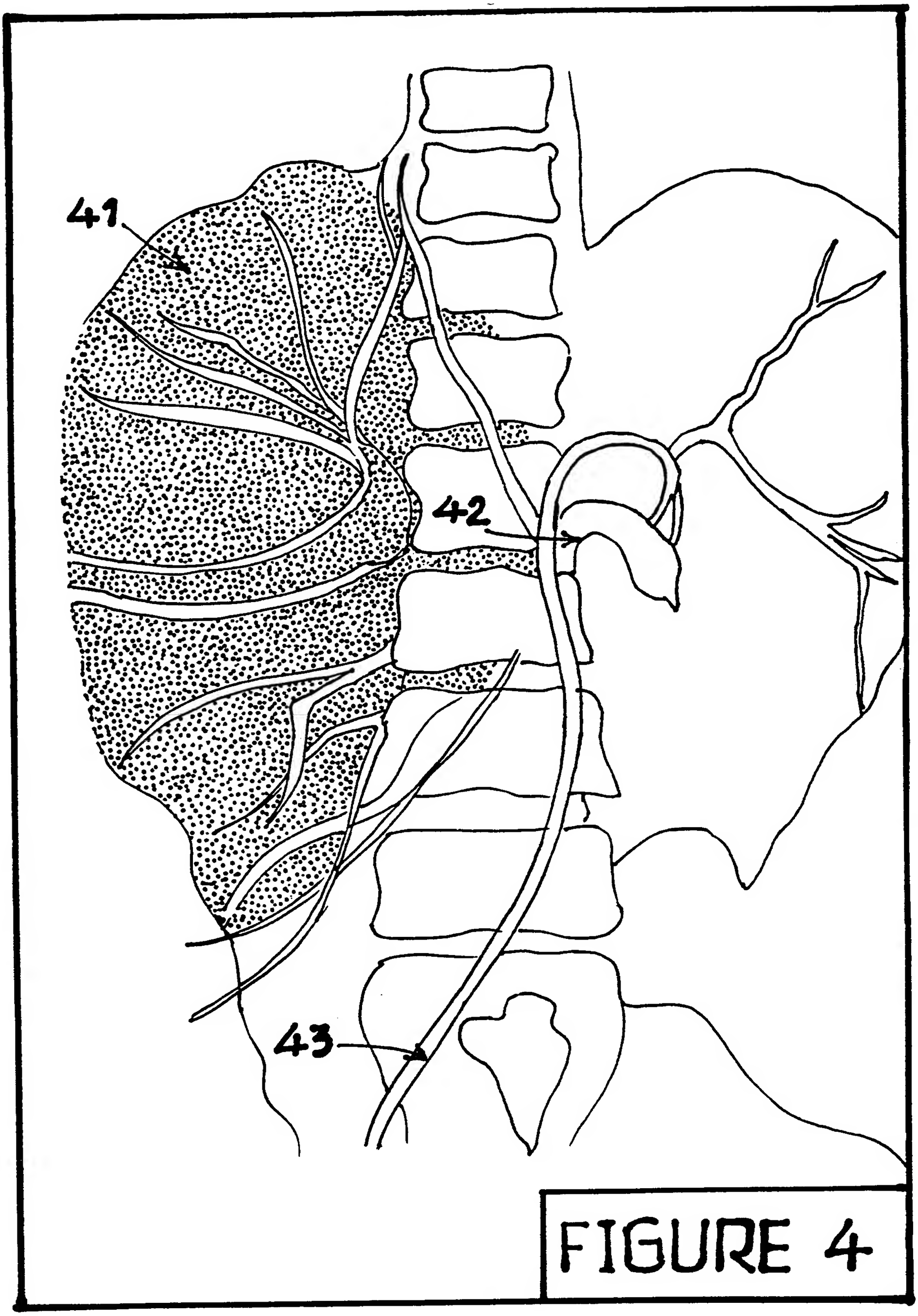

FIGURE 4

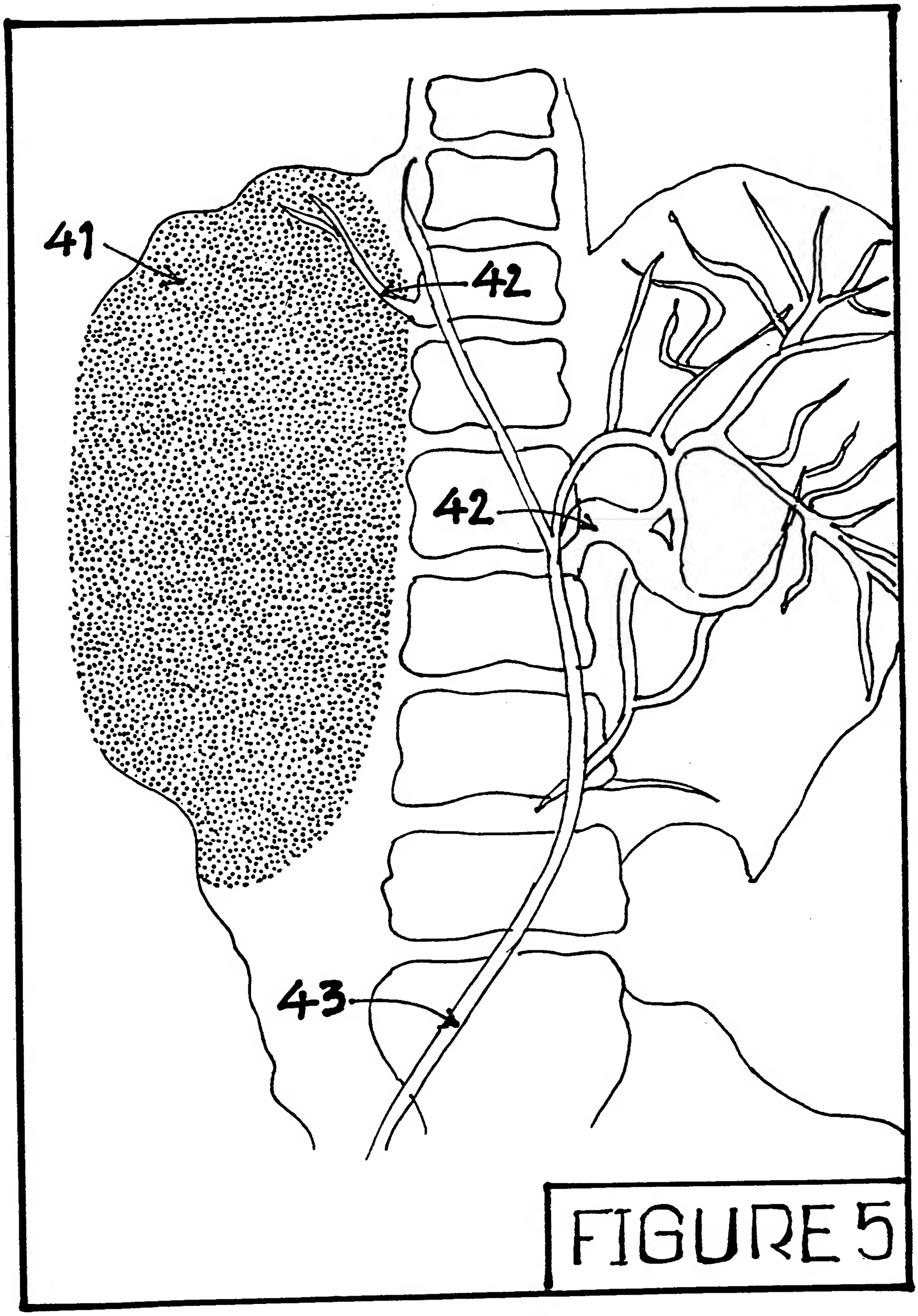

FIGURE 5

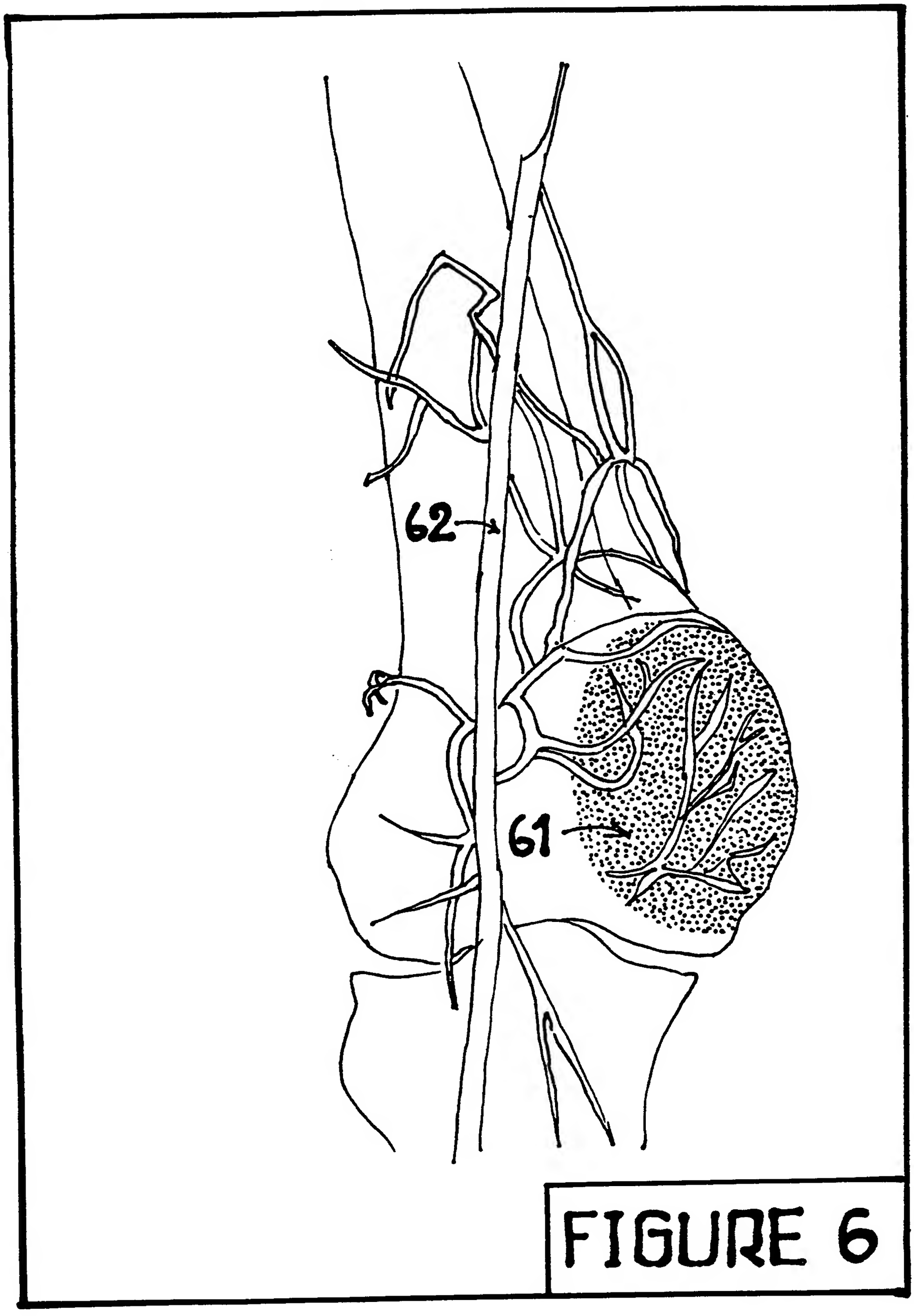

FIGURE 6

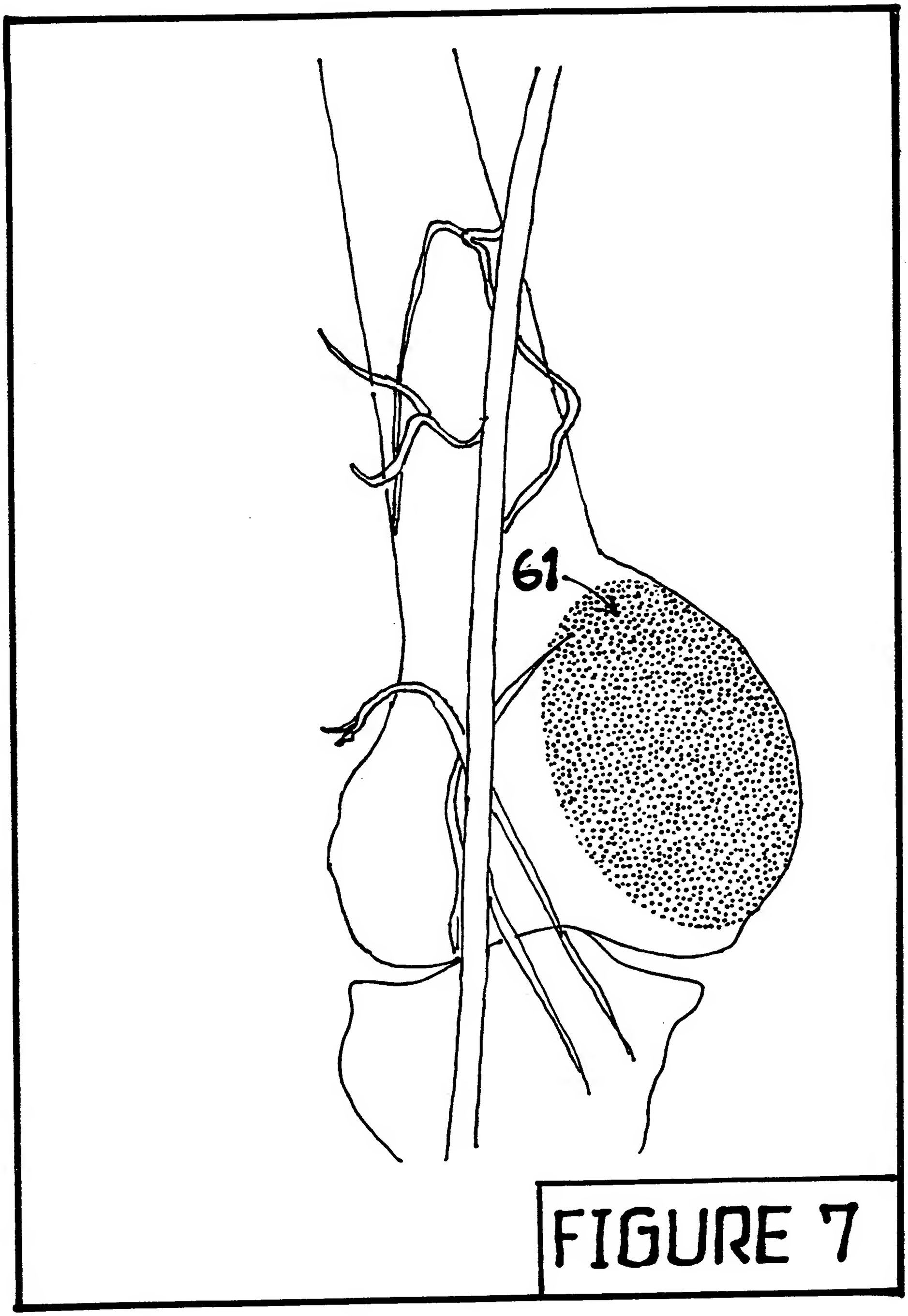

FIGURE 7

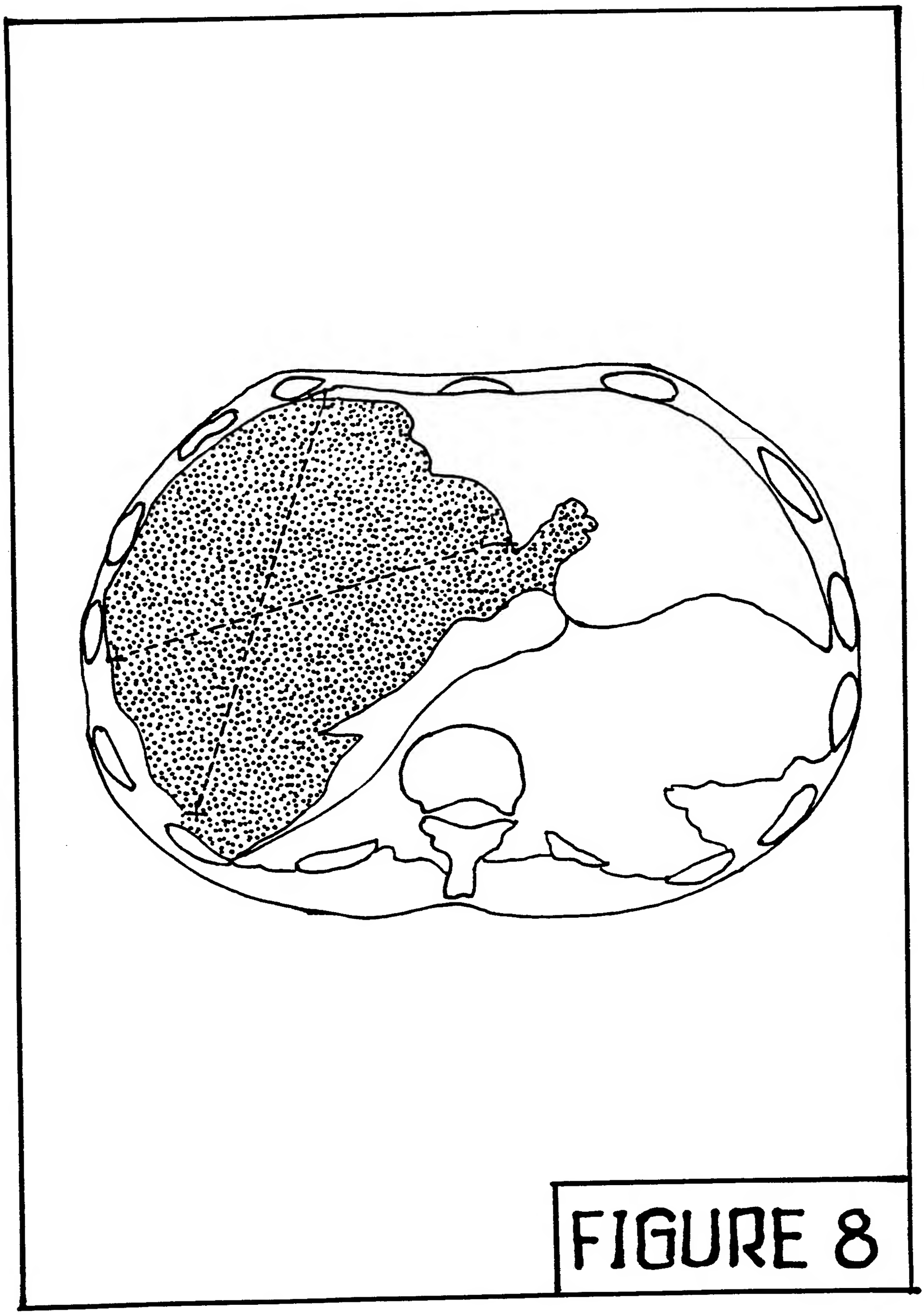

FIGURE 8

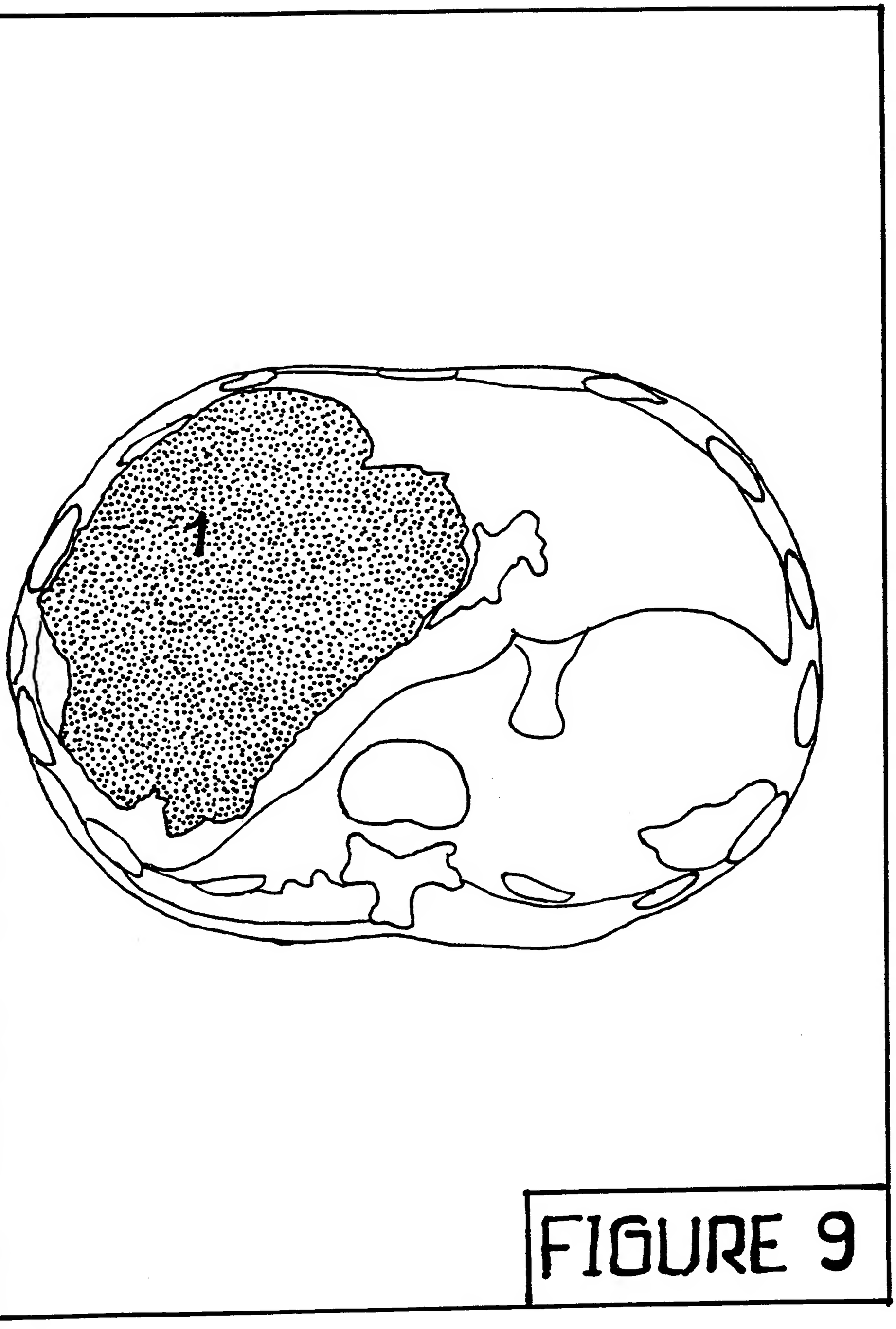

FIGURE 9

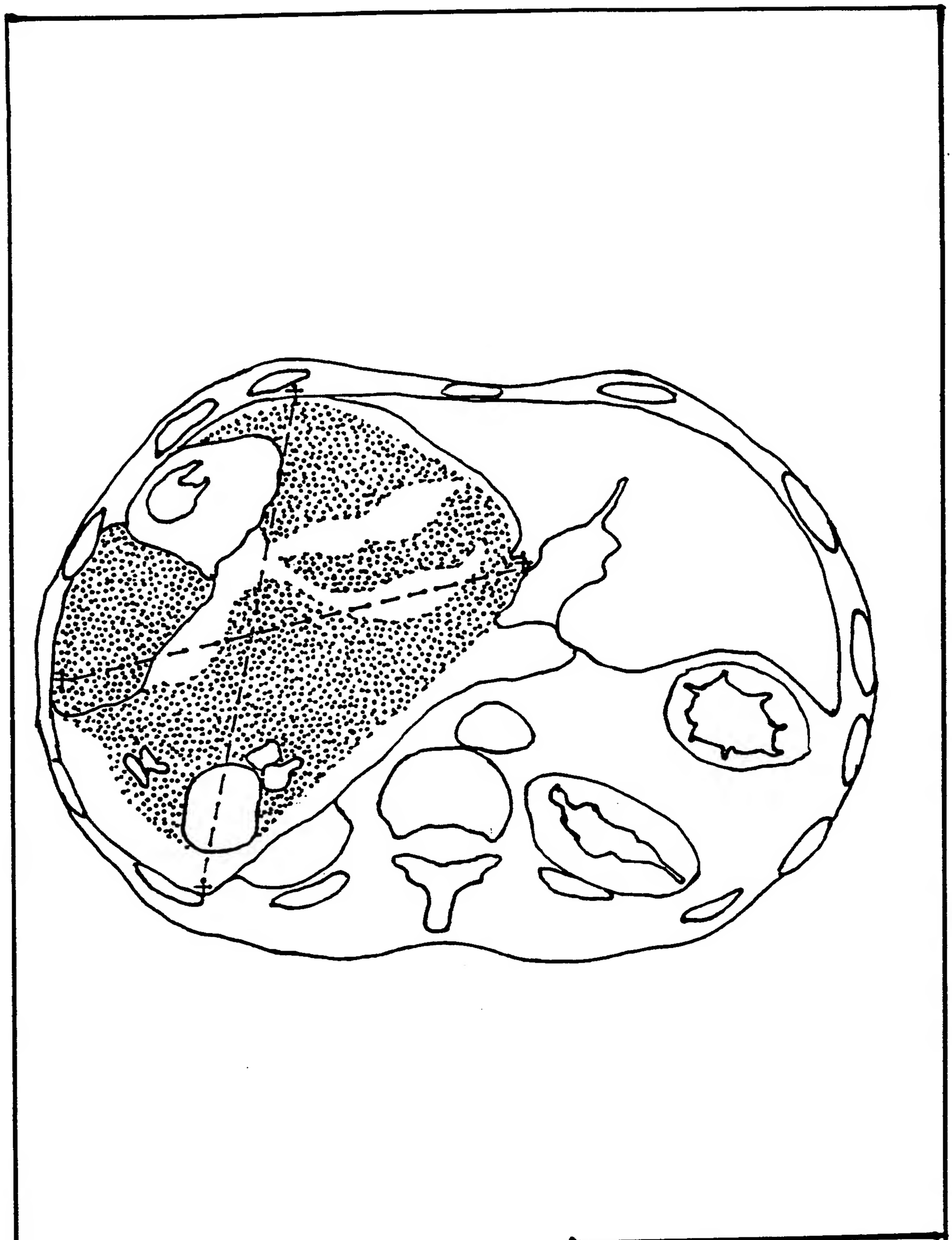

FIGURE 10

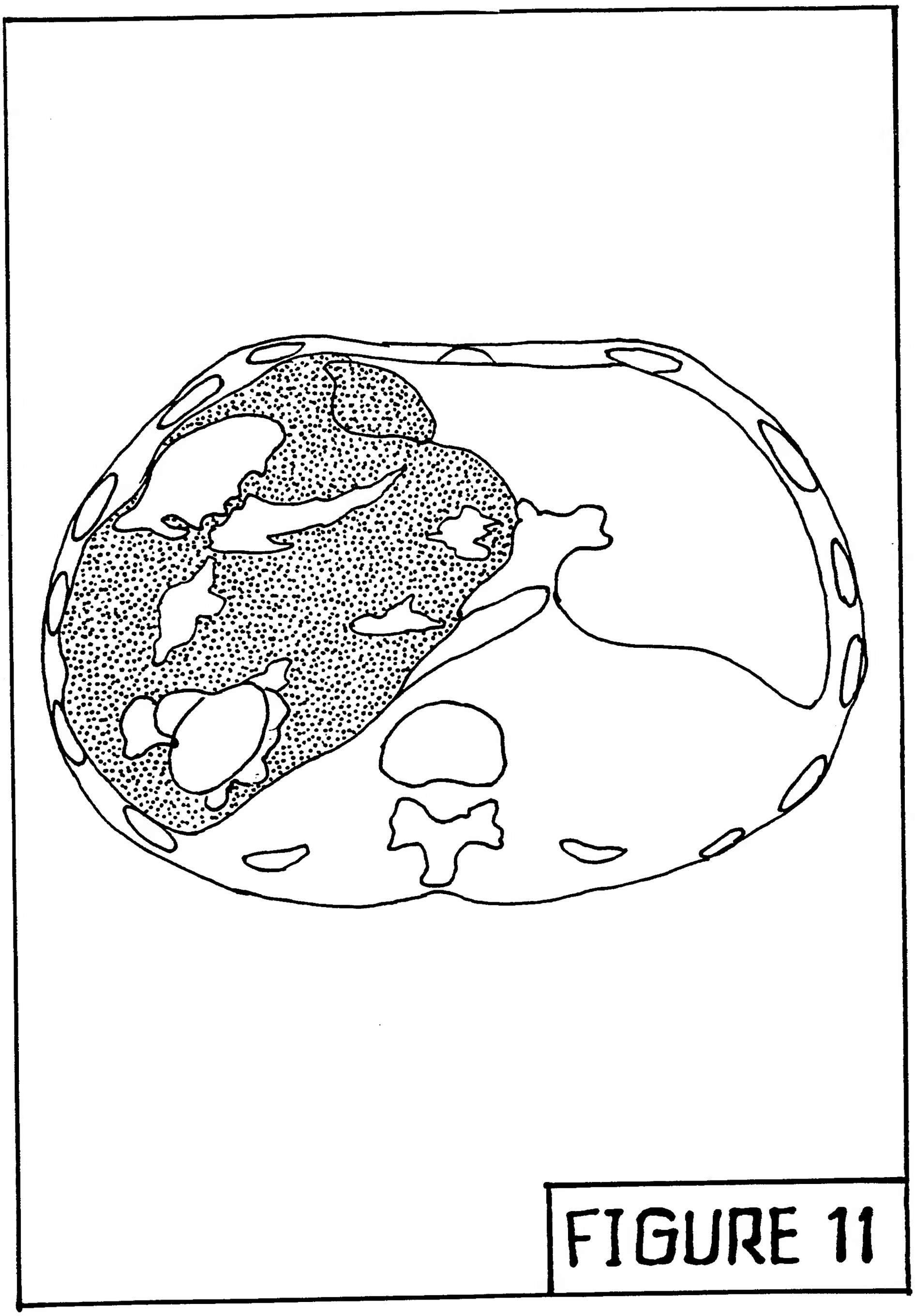

FIGURE 11

FIGURE 12

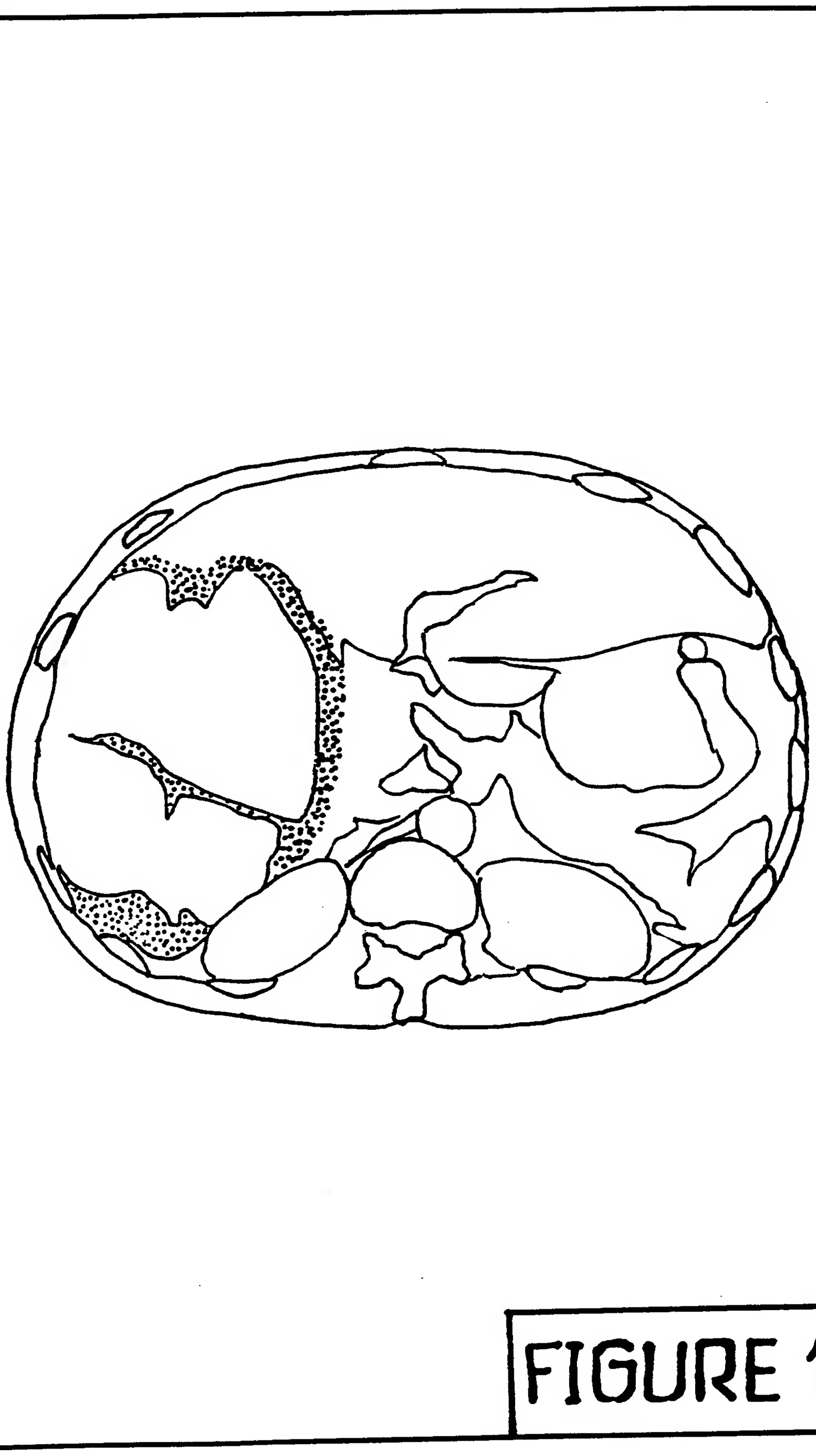

FIGURE 13

INTERNATIONAL SEARCH REPORT

Int. ational Application No
PCT/US 99/21711

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/20 A61K45/06 A61P35/00 A61K47/44 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, BIOSIS, MEDLINE, CHEM ABS Data, CANCERLIT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HAYASHI ET AL.: "Anticancer effect of free polyunsaturated fatty acids in an oily lymphographic agent following intrahepatic arterial administration to a rabbit bearing vx-2 tumor" CANCER RESEARCH, vol. 52, 1992, pages 400-405, XP000910143 cited in the application *see in particular the abstract; discussion at page 404 *	1
Y		1-8

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 August 2000	24.08.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Isert, B

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In. ational Application No
PCT/US 99/21711

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	OKAGAKI ET AL.: "Potentiation of the antitumor effect of aclarubicin on rat hepatoma model by hepatic arterially administered oily dosage forms" CHEMICAL & PHARMACEUTICAL BULLETIN, vol. 36, 1988, pages 3092-3097, XP000910165 *see in particular the abstract; page 3083 (2nd and 5h paragraph); and page 3094, Table 1 *	1
Y	---	1-6
Y	NAIDU ET AL.: "Intratumoral gamma-linoleic acid therapy of human gliomas" PROSTAGLANDINS LEUKOTRIENES AND ESSENTIAL FATTY ACIDS, vol. 45, 1992, pages 181-184, XP000909954 cited in the application *see in particular the abstract; page 183, left col., 2nd paragraph; discussion *	7,8

Y	DAS ET AL.: "Local application of gamma-linolenic acid in the treatment of human gliomas" CANCER LETTERS, vol. 94, no. 2, 1995, pages 147-155, XP000909873 *see in particular the abstract; last sentence at page 148; discussion *	7,8

Y	CAI ET AL.: "Inhibition of angiogenic factor- and tumour-induced angiogenesis by gamma linolenic acid" PROSTAGLANDINS LEUKOTRIENES AND ESSENTIAL FATTY ACIDS, vol. 60, no. 1, January 1999 (1999-01), pages 21-29, XP000909875 *see the summary; & page 28 *	1-8

Y	EP 0 585 057 A (SCOTIA HOLDINGS PLC) 2 March 1994 (1994-03-02) * see the claims; & example *	1-6

Y	GB 2 222 080 A (EFAMOL HOLDINGS) 28 February 1990 (1990-02-28) * see page 14; page 7, lines 1-15; page 10, lines 6-26 *	1-8

X	WO 98 09621 A (SCOTIA HOLDINGS PLC ;SCOTT CATHERINE ANN (GB); HORROBIN DAVID F (G) 12 March 1998 (1998-03-12) *see in particular claims 1-8; page 5 (ultimate paragraph) - page 9 *	9-14

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4

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Im..ational Application No
PCT/US 99/21711

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 795 909 A (SHASHOUA VICTOR E ET AL) 18 August 1998 (1998-08-18) cited in the application *see in particular claims 1-12; col. 8, lines 13-28; col. 47, lines 40-51; col. 46, lines 46-52 * ---	9-14
Y	WO 93 05774 A (WISCONSIN ALUMNI RES FOUND) 1 April 1993 (1993-04-01) *see in particular claims 1-6,9; page 13, line 13 - page 16; examples 1-6,10-11* ---	9-14
Y	PATENT ABSTRACTS OF JAPAN vol. 1996, no. 08, 30 August 1996 (1996-08-30) & JP 08 092129 A (KANAGAWA KAGAKU KENKYUSHO:KK), 9 April 1996 (1996-04-09) abstract ---	9-14
Y	PATENT ABSTRACTS OF JAPAN vol. 013, no. 069 (C-569), 16 February 1989 (1989-02-16) & JP 63 258816 A (NIPPON OIL & FATS CO LTD), 26 October 1988 (1988-10-26) abstract -----	9-14

4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/21711

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-8

   Method for treating tumor by intra-arterial or intra-tumoral injection of PUFAs

2. Claims: 9-14

   Method for treating a tumor by combining PUFAs with anti-cancer drugs

INTERNATIONAL SEARCH REPORT

Information on patent family members

Im. .ational Application No
PCT/US 99/21711

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